IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Confirmation No.	**:**	**5916**
Appln. No.	**:**	10/675,172
Applicant	**:**	Stephen Donovan
Filed	**:**	09/29/2003
TC/A.U.	**:**	1645
Examiner	**:**	Ford, Vanessa L.
Docket No.	**:**	17510DIV2 (BOT)
Customer No.	**:**	51957
Title	**:**	Transdermal Botulinum Toxin Administration

APPELLANT'S BRIEF (37 CFR § 1.192)

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This brief is in furtherance of the Notice of Appeal, filed in this case on November 19, 2009.

The fees required under § 1.17, and any required petition for extension of time for filing this brief and fees therefor, are hereby authorized to be withdrawn from the deposit account No. 50-3207.

Respectfully submitted,

Dated: December 30, 2009

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REAL PARTY IN INTEREST

The inventor, Stephen Donovan, has assigned his entire interest in this patent application to Allergan, Inc. via an assignment document executed on July 11, 2002, and recorded with the United States Patent and Trademark Office on July 11, 2001 at Reel/Frame: 013111/0469.

Allergan, Inc. is therefore, the owner of this patent application and the real party in interest in this appeal.

RELATED APPEAL AND INTERFERENCES

There are no related appeals or interferences.

STATUS OF THE CLAIMS

Claims 22-28, 36 and 37 were finally rejected in the Final Office Action dated June 23, 2009. Claims 22-28, 36 and 37 are pending and currently presented on appeal.

Claims 1-21 were cancelled in the Preliminary Amendment filed September 23, 2003. Claims 31-35 were cancelled in the response filed May 25, 2005. Claims 29 and 30 were cancelled, and claims 36 and 37 were added in the response filed November 13, 2006. No claims have been allowed.

STATUS OF AMENDMENTS

No amendments have been made subsequent to the Final Office Action mailed on June 23, 2009.

SUMMARY OF CLAIMED SUBJECT MATTER

One independent claim and eight dependent claims are currently being presented on appeal.

Claim 22 is an independent claim directed to a method of reducing neurotransmitter release in a subdermal structure of a patient comprising (a) non-chemically disrupting and reducing the impermeability of the stratum corneum, (b) applying a fluid to the patient's skin, (c) applying a transdermal patch to the disrupted stratum corneum, the patch comprising (i) a pharmaceutical composition comprising botulinum toxin in a dried state, an enhancing agent that is mixable with and facilitates the transdermal administration of botulinum toxin in a bioactive form to a subdermal target site without being administered to the patient's circulatory systems, and (ii) an adhesive layer to removably secure the transdermal patch to the skin, where the pharmaceutical composition is incorporated into the adhesive layer, and (d) solubilizing the botulinum toxin with the fluid, permitting diffusion of the toxin from the adhesive layer into the patient's skin. Support for claim 22 can be found, for example, at page 19, lines 19 to 25; page 20, lines 6 to 14; and page 20, line 29 to page 21, line 4.

Claim 23 is a dependent claim drawn to the method of claim 22, where the stratum corneum is disrupted by abrasively removing the stratum corneum. Support for claim 23 can be found, for example, at page 21, lines 2 to 6.

Claim 24 is a dependent claim drawn to the method of claim 22, where the stratum corneum is disrupted by applying an adhesive material to the patient's skin and then removing the adhesive material. Support for claim 24 can be found, for example, at page 21, lines 2 to 6.

Claim 25 is a dependent claim drawn to the method of claim 22, where the stratum corneum is disrupted by applying ultrasound at a frequency between 20 kHz to 1 MHz at an intensity that does not permanently damage the patient's skin. Support for claim 24 can be found, for example, at page 21, lines 6 to 9; and page 29, lines 26 to 27.

Claim 26 is a dependent claim drawn to the method of claim 22, where the stratum corneum is disrupted by passing an electrical current from a first point to a second point. Support for claim 26 can be found, for example, at page 21, lines 9 to 13.

Claim 27 is a dependent claim drawn to the method of claim 26, where the electrical current creates a plurality of pores in the stratum corneum, enhancing the passage of botulinum toxin to the subdermal structures. Support for claim 27 can be found, for example, at page 21, lines 9 to 13.

Claim 28 is a dependent claim drawn to the method of claim 22, where the botulinum toxin is selected from serotypes A, B, C, D, E, F and G. Support for claim 28 can be found, for example, at page 19, lines 6 to 9.

Claim 36 is a dependent claim drawn to the method of claim 22, where the fluid further includes an enhancing agent. Support for claim 36 can be found, for example, at page 19, lines 1 to 6.

Claim 37 is a dependent claim drawn to the method of claim 25, where the ultrasound is delivered prior to application of the botulinum toxin to the skin. Support for claim 37 can be found, for example, at page 30, lines 17 to 22.

GROUNDS FOR REJECTION TO BE REVIEWED ON APPEAL

Claims 22-24, 28 and 36 were finally rejected pursuant to 35 U.S.C. §103(a) as unpatentable over Pearce et al. (U.S. Patent No. 6,087,327), in view of Mohr et al. (U.S. Patent No. 5,591,767), and further in view of Singer et al. (Acad. Emerg. Med., Nov. 1998; 5(11), p. 1051-6; Abstract only).

Claims 25 and 27 were finally rejected pursuant to 35 U.S.C. §103(a) as being unpatentable over Pearce et al., Mohr et al., Singer et al. as applied to claims 22-24, 28 and 35, and further in view of Mitragotri et al. (Science, Vol. 269, August 11, 1995).

Claims 26 and 27 were finally rejected pursuant to 35 U.S.C. §103(a) as being unpatentable over Pearce et al., Mohr et al., Singer et al., Mitragotri et al. as applied to claims 22-25, 28 and 36-37, and further in view of Yuzhakov et al. (U.S. Patent No. 6,565,532).

ARGUMENTS

I. Introduction

Claims 22-28, 36 and 37 are pending in this application. Three rejections remains in this application and are currently being presented for review on appeal. First, Claims 22-24, 28 and 36 are rejected under 35 U.S.C. § 103(a) as unpatentable over Pearce et al (U.S. Patent No. 6,087,327 issued July 11, 2000) ("Pearce"), in view of Mohr et al. (U.S. Patent No. 5,591,767 issued January 7, 1997) ("Mohr") and further in view of Singer et al. (Acad Emerg. Med, Nov. 1998; 5(11), p.1051-6; Abstract only) ("Singer"). Second, Claims 25 and 37 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Pearce, Mohr, Singer, and further in view of Mitragotri et al. (Science, Vol. 269, Aug. 11, 1995) ("Mitragotri"). And lastly, Claims 26 and 27 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Pearce, Mohr, Singer, and Mitragotri as applied to claims 22-25, 28 and 36-37 above, and further in view of Yuzhakov et al. (U.S. Patent No. 6,565,532 published May 20, 2003) ("Yuzhakov"). Appellant respectfully disagrees with the aforementioned rejections and requests the Board reverses these finding for the following reasons.

II. <u>35 U.S.C. § 103(a) Rejections Should Be Reversed Because the Office Has Not Met Its Burden of Establishing a *Prima Facie* Case of Obviousness</u>.

Obviousness is a question of law based on underlying factual inquiries. MPEP § 2141(II). Support for any 35 U.S.C. § 103 rejection must include a "clear articulation of the reason(s) why the claimed inventions would have been obvious" to one of ordinary skill in the art at the time the inventions were made. MPEP § 2142, *citing KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007). The Office bears the initial burden of factually supporting a *prima facie* conclusion of obviousness, and if the Office does not meet this burden, Appellants are "under no obligation to submit evidence of nonobviousness." MPEP § 2142; *In re Oetiker*, 977 F.2d 1443, 1445 (Fed. Cir. 1992).

Here, the Office has failed to provide factual evidence or clearly articulated reasons explaining why the claimed invention would have been obvious to a person of

ordinary skill in the art at the time of the invention. Thus, Appellant submits that without conceding to the propriety of the asserted combinations, the combinations of Pearce, Mohr, Singer, Mitragotri and Yuzhakov, in view of the knowledge of one ordinarily skilled in the art, are insufficient to present *prima facie* obviousness.

A. 35 U.S.C. § 103(a) Rejection of Claims 22-24, 28 and 36.

Claims 22-24, 28 and 36 are rejected under 35 U.S.C. § 103(a) as unpatentable over Pearce et al (U.S. Patent No. 6,087,327 issued July 11, 2000) ("Pearce"), in view of Mohr et al. (U.S. Patent No. 5,591,767 issued January 7, 1997) ("Mohr") and further in view of Singer et al. (Acad Emerg. Med, Nov. 1998; 5(11), p.1051-6; Abstract only) ("Singer"). According to the Office, it would have been *prima facie* obvious to

> modify the method of reducing the neurotransmitter release in a subdermal structure of a patient as taught by Pearce to include the transdermal patch of Mohr et al which incorporates drugs and skin enhancers (e.g. botulinum toxin and the enhancing agent) into the adhesive layer and non-chemically disrupting the stratum corneum of the patient's skin to reduce the impermeability of the stratum corneum as taught by Singer et al. because Mohr et al has demonstrated that this design of transdermal patch is simple but effective in delivering drugs to the skin and Singer et al teach that tape stripping enhances absorption of drugs into the skin.

(June 23, 2009 Office Action, p.3-4). Appellant respectfully asserts that the Office has misinterpreted and broadly applied the teachings of the cited art, thus inappropriately concluding the present claims as obvious.

When assessing the differences between a claimed invention and the teachings of the prior art, the Office must remember that "virtually all [inventions] are combinations of old elements" and therefore, care must be taken to <u>not</u> break an invention into its component parts, find references containing each part, and conclude the invention obvious. *Ruiz*, 357 F.3d at 1276, 69 USPQ2d at 1690, *citing Envtl. Designs, Ltd. v. Union Oil Co.*, 713 F.2d 693,698 (Fed Cir. 1983). In essence, this form of analysis constitutes hindsight reasoning and uses the present invention as a road map to find prior art components. *Id.* To ensure that hindsight reasoning is avoided, the Federal Circuit has repeatedly required a showing that a skilled artisan "confronted by the same

problems as the inventor and with no knowledge of the claimed invention, would select the various elements from the prior art and combine them in the claimed manner." *Id.*, *citing In re Rouffet*, 149 F.3d 1350, 1355-56 (Fed. Cir. 1996) (emphasis added).

Here, the Office has separated the method of claim 22 into its component parts, cited nonanalogous references containing some of the component parts, and thus concluded the invention obvious. The Office has not shown that a person of ordinary skill in the art the time, with no knowledge of the claimed compositions, would have arrived at the claimed compositions from the teachings of Pearce, Mohr and Singer.

Pearce teaches the use of botulinum toxin compositions for localized denervation. Pearce generally discloses topical administration and transdermal diffusion as contemplated routes of administration. Pearce does not disclose enhancing agents or specific methods of facilitating the delivery of the botulinum toxin compositions to tissues in need thereof. Given the immense size of botulinum toxin complex (300-900 kDa) (Specification, p. 5, ln. 3-12) and the inherent problems with transdermal drug delivery (e.g. loss of bioactivity, impermeability of skin, etc.) one of ordinary skill in the art at the time would not have found the presently disclosed methods of transdermally administering a botulinum toxin composition obvious in light of the teachings of Pearce, as combined with the teachings of the other cited references and the knowledge of one ordinarily skilled in the art at the time.

Mohr teaches a patch for the transdermal delivery of ketorolac, a non-steroidal anti-inflammatory agent with analgesic properties. (Mohr, col.1, ln.15-16). Mohr does not teach methods for the transdermal delivery of botulinum toxin compositions. Botulinum toxin has a molecular mass of approximately 150,000 Da, or 150 kDa. (Pearce col. 1, ln. 55-56; Specification, pg. 5, ln. 1-2). Ketorolac tromethamine, a pharmaceutically acceptable salt of Ketorolac, has a molecular weight of 376.41 g/mol (approximately 376.41 Da). (Mohr, col. 5, ln. 63-65). One of ordinary skill in the relevant art would not have found it obvious to employ the same method used to facilitate transdermal delivery for a 376.41 g/mol molecule to facilitate the transdermal delivery of botulinum toxin, a molecule almost 500 times the size.

Moreover, "A prior art reference must be considered in its entirety, i.e., as a whole, including portions which would lead away from the claimed invention." *W.L. Gore & Associates, Inc. V. Garlock, Inc.* 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). A reference is said to teach away if a person of ordinary skill, upon reading the reference, "would be led in a direction divergent from the path that was taken by the applicant." *In re Gurley*, 27 F.3d 551, 552, 31 USPQ2d 1130, 1131 (Fed. Cir. 1994). In the present case, Mohr specifically teaches that a transdermal patch is "particularly beneficial when it is ***desired to maintain a constant blood level*** of drug in the patient for extended periods of time." (Mohr, col. 1, ln. 50-54 (emphasis added)). Botulinum toxin is an extremely lethal biological agent and an ordinary skilled artisan would not seek to utilize methods of toxin delivery that would maintain constant blood levels of the toxin for extended periods of time. In fact, the present specification and claims clearly denote that the present invention ***excludes*** systemic administration of the neurotoxin. (Specification, pg. 15, ln. 19-30; and claim 22). One of ordinary skill in the art at the time of the invention, reading the teachings of Mohr, would be led in a direction divergent from the path taken by Appellant and thus, would not find the present methods obvious.

Singer does not cure the deficiencies of Pearce and Mohr. Singer teaches the use of cutaneous tape stripping for enhancing topical absorption of medications. (Singer, Abstract, p.1051, col. 1-2). The methods taught in Singer, similar to those taught in Mohr, focus on enhanced absorption of relatively small molecules such as lidocaine and prilocaine. (Singer, p.1051, col. 2). One of ordinary skill in the relevant art would not have found it obvious to employ the same method used to facilitate enhanced topical absorption of a small molecule such as lidocaine or prilocaine to facilitate enhanced topical absorption of botulinum toxin.

It is impermissible for the Office to use the present claims "as an instruction manual or a template to piece together the teachings of the prior art so that the claimed invention is rendered obvious. *In re Fritch*, 972 F.2d 1260, 1264 (Fed. Cir. 1992). Here, the Office has done exactly that. The Office has used the present claims as a road map

to piece together dissimilar, unrelated teachings in an effort to reconstruct the present invention. The Office fails to identify what explicit or implicit motivation, or general knowledge available to one ordinarily skilled in the art at the time of the invention would have led the artisan to combine or modify the cited references, as combined by the Office, to arrive at the present methods.

The test for obviousness is what the combined teachings would have suggested to an ordinary skilled artisan. *In re Keller*, 642 F.2d 413, 425 (CCPA 1981). The combined teachings of Pearce, Mohr and Singer do not suggest a method for reducing neurotransmitter release in a subdermal structure of a patient comprising disrupting the stratum corneum and applying a transdermal patch including a botulinum toxin composition to the skin of the patient. The Office has not identified a reason that would have prompted one of ordinary skill in the art to combine the elements as the claimed invention does and therefore, has not established a *prima facie* case of obviousness. *See Takeda Chem. Indus. Ltd. v. Alphapharm Pty., Ltd.*, 492 F.3d 1350, 1356-1357 (Fed. Cir. 2007).

Moreover, since the combination of Pearce, Mohr and Singer does not teach or suggest all the limitations claimed in claim 22 and "[a] claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers," it necessarily follows that the combined references do not disclose the methods of dependent claims 23, 24, 28 and 36. *See* 35 U.S.C. § 112. As such, Appellant respectfully requests the Board reverse the 35 U.S.C. § 103 (a) rejection of claims 22-24, 28 and 36.

B. 35 U.S.C. § 103(a) Rejection of Dependent Claims 25 and 37.

Dependent Claims 25 and 37 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Pearce, Mohr, Singer, and further in view of Mitragotri et al. (Science, Vol. 269, Aug. 11, 1995) ("Mitragotri"). According to the Office, it would have been *prima facie* obvious to modify the teachings of Pearce, Mohr, Singer and Mitragotri because a skilled artisan would expect that "a transdermal patch comprising botulinum

toxin and an enhancing agent within the adhesive layer and using ultrasound to enhance delivery of the botulinum toxin to the skin would be an effective way to facilitate the delivery of active agents such as botulinum toxin to a subdermal target of a patient's skin that has been non-chemically disrupted by tape stripping, thereby reduce (*sic.*) neurotransmitter release in a subdermal structure of the patient." (June 23, 2009 Office Action, p.11). However, the Office has misinterpreted and misapplied the teachings of the cited art.

Claims 25 depends from independent claim 22 and claims a method in which the stratum corneum is disrupted by ultrasound frequencies between 20 kHz to 1 MHz. Claim 37 further limits claim 25 to a method wherein the ultrasound is delivered prior the to the application of botulinum toxin. Contrary to the Office's assertions, neither claim 25 nor claim 37 include a limitation in which the patient's skin is disrupted by tape stripping. Further, Mitragotri's teachings focus on the transdermal delivery of insulin, a blood-based protein, and methods of increasing blood insulin concentrations. (Mitrgotri, p. 851, col. 2; p. 852, col. 1). Similar to Mohr, Mitragotri teaches that transdermal administration has the "benefit" of maintaining high blood concentrations of the therapeutic agent being administered. (Mitragotri, p. 850, col. 1). One of ordinary skill in the art at the time of the present invention, familiar with the teachings of Mohr and Mitragotri, would not have found it obvious to transdermally administer botulinum toxin to a subdermal target site when seeking to avoid administration to the patient's circulatory system.

To support its conclusions and it *prima facie* case of obviousness, the Office must clearly articulate a reason that would have prompted one ordinarily skilled in the relevant art to combine the elements the way the claimed invention does. *Takeda Chem. Indus., Ltd. v. Alphapharm Pty., Ltd.*, 492, F.3d 1350, 1356-1357 (Fed. Cir. 2007). Here, the Office fails to provide a logical rationale explaining the suggestion or motivation that would have prompted such an artisan to combine the elements the way the claimed methods do. As a result, the Office has not met its *prima facie* burden of going forward and Appellant respectfully requests the Board reverse this rejection.

C. 35 U.S.C. § 103(a) Rejection of Claims 26 and 27.

Dependent claims 26 and 27 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Pearce, Mohr, Singer, and Mitragotri as applied to claims 22-25, 28 and 36-37 above, and further in view of Yuzhakov et al. (U.S. Patent No. 6,564, 532 published May 20, 2003) ("Yuzhakov"). According to the Office, it would have been *prima facie* obvious at the time the invention was made to modify the methods of "reducing neurotransmitter release" as taught by Pearce "by using the microneedle array comprising electrodes that apply electric potential to a patient's skin as taught by Yuzhakov, the ultrasound applied to a patient's skin according to Mitragotri et al. would enhance the delivery of the pharmaceutical compositions (e.g. botulinum toxin and an enhancing agent) incorporated within the adhesive layer of the transdermal patch (as taught by the combination of Mohr et al.) into the subdermal layers of a patient's skin which has been non-chemically disrupted as taught by Singer et al. " (June 23, 2009 Office Action, p. 14). However, again, the Office has misinterpreted and misapplied the teachings of the cited art.

Claim 26 is dependent claim drawn to the method of claim 22, where the stratum corneum is disrupted by passing an electrical current from a first point to a second point on the patient's skin. Claim 27 further limits claim 26 to a method where the electric current creates a plurality of pores in the stratum corneum to enhance the passage of botulinum toxin to the subdermal structures. Contrary to the Office's assertions, neither claim 26 nor claim 27 specifically include a limitation in which the patient's skin is disrupted by tape stripping or by ultrasound applied to a patient's skin. Further, Yuzhakov teaches the use of ***microneedles*** to facilitate ***intracutaneous*** drug delivery. (Yuzhakov, col. 2, ln. 61 to col. 3, ln. 8). The microneedles are designed to penetrate the stratum corneum to facilitate drug delivery or fluid sampling. (Yuzhakov, col. 4, ln.17-19). While certain embodiments taught by Yuzhakov utilize electrophoresis in combination with the microneedle apparatus, the stratum corneum is disrupted by the microneedle piercing, not the applied electrical field. (Yuzhakov, col. 5, ln 44-46).

Prima facie obviousness can only be established if all the claim limitations are taught or suggested in the prior art. *In re Royka*, 490 F.2d 981. Here, the combination of Yuzhakov and the cited art does teach or suggest a method of reducing neurotransmitter release in a subdermal structure of a patient where an electrical current is used to disrupt and create a plurality of pores in the stratum corneum. Thus, the proposed combination of Pearce, Mohr, Singer, Mitragotri and Yuzhakov does not teach or suggest the present claims. Additionally, the Office has failed to present a convincing line of reasoning as to why a skilled artisan would have found the claimed invention obvious in light of the cited teachings. As a result, the Office has not met its *prima facie* burden of going forward and Appellant respectfully requests the Board reverse this rejection.

III. Conclusion

In summary, Appellant asserts that the Office's 35 U.S.C. § 103(a) obviousness rejections are improper because the Office has failed to meet its initial burden of establishing a *prima facie* case of obviousness, as detailed above.

Accordingly, Appellant asks the Board to reverse the aforementioned rejections. Appellant further asks the Board to direct the Office to issue a Notice of Allowance for claims 22-28, 36 and 37, and the claims be allowed to proceed to issue.

Appellant files herewith a deposit account authorization for payment of the fee associated with the filing of this Appeal Brief. If any other fee is due, the Commissioner is authorized to charge any such fee to deposit account number 01-0885.

CLAIM APPENDIX

Listing of Claims:

1-21. (Cancelled)

22. (Previously Presented) A method of reducing neurotransmitter release in a subdermal structure of a patient, the method comprising the steps of:

(a) non-chemically disrupting the stratum corneum of the patient's skin to reduce impermeability of the stratum corneum:

(b) applying a fluid to the patient's skin;

(c) applying a transdermal patch to the skin of the patient in an area that had the stratum corneum disrupted in step (a), the transdermal patch comprising;

(i) a pharmaceutical composition comprising a stabilized botulinum toxin provided in a dried state and an enhancing agent that is mixable with the stabilized botulinum toxin provided in a dried state and facilitates transdermal administration of a botulinum toxin in a bioactive form to a subdermal target site of a human patient without being administered to the patient's circulatory systems; and

(ii) an adhesive layer disposed to one side of the transdermal patch to removably secure the patch on the patient's skin;

Wherein the pharmaceutical composition is incorporated into the adhesive layer; and

(d) solubilizing the botulinum toxin provided in the dry state with the fluid, wherein solubilization of the botulinum toxin permits diffusion of the

botulinum toxin from the adhesive layer into the patient's skin thereby reducing neurotransmitter release in a subdermal structure.

23. (Original) The method of claim 22, wherein the stratum corneum is disrupted by abrasively removing the stratum corneum.

24. (Original) The method of claim 22, wherein the stratum corneum is disrupted by applying an adhesive material to the patient's skin, and removing the adhesive material applied thereto.

25. (Previously Presented) The method of claim 22, wherein the stratum corneum is disrupted by applying ultrasound at a frequency between 20Khz to 1 MHz at an intensity that does not permanently damage the patient's skin.

26. (Original) The method of claim 22, wherein, the stratum corneum is disrupted by passing an electrical current from a first point on the patient's skin to a second point on the patient's skin.

27. (Original) The method of claim 26, wherein the electrical current is passed to create a plurality of pores in the stratum corneum to enhance passage of botulinum toxin to the subdermal structures.

28. (Original) The method of claim 22, wherein the botulinum toxin is selected from the group of botulinum toxins consisting of types A, B, C, D, E, F and G.

29-35. (Cancelled)

36. (Previously Presented) The method of claim 22, wherein said fluid further includes an enhancing agent.

37. (Previously Presented) The method of claim 25, wherein the ultrasound application is delivered prior to application of the botulinum toxin to the skin.

EVIDENCE APPENDIX

1. Mitragotri et al., Science, Vol. 269, Aug. 11, 1995.

2. Singer et al., Acad. Emerg. Med., Dov. 1998; 5(11), p.1051-6; Abstract only.

RELATED PROCEEDINGS APPENDIX

None

described (19). Immunofluorescence of α-rENaC (7) used e polyclonal antibody against a glutethione-S-transferase fusion protein containing the first 76 amino ecids of the NH_2-terminus (29) end CFTR protein-1468 (30), following the protocol of Grubb et al. (15). Cells on coeted cover slips were fixed in acetone at 20°C for 10 min and incubated in primary antibodies for 90 min at room temperature followed by fluorescein isothiocyanate (FITC)– and Texas Red–conjugated secondary antibodies for 30 min. Parent MDCK cells were used as control. Similar results were obtained for the rENaC subunit.
17. H. H. Ussing, *Physiol. Rev.* **29**, 127 (1949).
18. C. U. Cotton, M. J. Stutts, M. R. Knowles, J. T. Gatzy, R. C. Boucher, *J. Clin. Invest.* **79**, 80 (1987).
19. M. J. Stutts *et al.*, *J. Biol. Chem.* **268**, 20653 (1993).
20. We studied 3T3 cells on the stage of en inverted microscope at 22°C by the techniques of Hamill *et al.* (31). Pipettes were filled with Cl^--free buffer that contained 100 mM Na aspartate, 2 mM $MgSO_4$, 2 mM Na_2ATP, and 5 mM *N*-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid (TES) (pH 7.2). Cl^--free extracellular buffer contained 160 mM Na gluconate, 2 mM $MgSO_4$, 1 mM $CaSO_4$, and 5 mM TES (pH 7.2). [See (32) for other details.] Solutions conteining emiloride (final concentration 10^{-5} M) and cpt-cAMP (5×10^{-4} M)/forskolin (1×10^{-5} M) were added by e 1:2 dilution of the extracellular bath.
21. Swiss 3T3 fibroblasts stebly expressing the human CFTR gene (32) or an inactive interleukin-2 receptor (33) were infected with a retrovirus expression vector encoding the α-, β-, and γ-rENaC subunits. The three subunits were expressed from the viral long terminal repeat on a tricistronic mRNA in the order (5' to 3') α, β, γ. Internal ribosomal entry site sequences from encephalomyocarditis virus end poliovirus were included 5' of the β and γ subunit sequences, respectively, to facilitate translation. Immediately 3' to the γ eubunit, an SV40 promoter was included to drive transcription of a puromycin-selectable marker. Fibroblasts were exposed to virus (10^2 CFU/ml) in polybrene (8 μg/ml) and were selected in puromycin (1 μg/ml). Resistant colonies were expanded and expression of α-, β-, and γ-rENaC subunits was determined by Northern (RNA) blot and protein immunoblot analyses. Immunocytochemistry was as described in Fig. 2.
22. D. A. Ausiello, J. L. Stow, H. F. Cantiello, J. B. de Almeida, D. J. Benos, *J. Biol. Chem.* **267**, 4759 (1992).
23. I. L. Reisin *et al.*, *ibid.* **269**, 20584 (1994); E. M. Schwiebert, T. Flotte, G. R. Cutting, W. B. Guggino, *Am. J. Physiol.* **266**, C1464 (1994).
24. S. Laferte end L. C. Loh, *Biochem. J.* **283**, 193 (1992).
25. R. M. Rios, C. Celeti, S. M. Lohmann, M. Bornens, G. Keryer, *EMBO J.* **11**, 1723 (1992).
26. B. Serkadi *et al.*, *J. Biol. Chem.* **267**, 2087 (1992).
27. N. J. Willumsen, C. W. Davis, R. C. Boucher, *Am. J. Physiol.* **256**, C1033 (1989).
28. B. R. Grubb, R. N. Vick, R. C. Boucher, *ibid.* **266**, C1478 (1994).
29. C. M. Canessa, A. Merillet, B. C. Rossier, *ibid.* **267**, C1682 (1994).
30. J. A. Cohn, O. Melhus, L. J. Page, K. L. Dittrich, S. R. Vigna, *Biochem. Biophys. Res. Commun.* **181**, 36 (1991).
31. O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981).
32. M. J. Stutts *et al.*, *J. Biol. Chem.* **269**, 8667 (1994).
33. J. C. Olsen *et al.*, *Hum. Gene Ther.* **3**, 253 (1992).
34. We thank P. Blonigen, H. Suchindran, U. S. Rao, and H. Ye for technical contributions and L. Brown for editorial assistance. Supported by NIH grants HL 34322, HL 42384, and CFF R026 and by Swiss Nationel Foundation grant 31-33598.92.

23 February 1995; accepted 19 June 1995

Ultrasound-Mediated Transdermal Protein Delivery

Samir Mitragotri, Daniel Blankschtein,* Robert Langer*

Transdermal drug delivery offers a potential method of drug administration. However, its application has been limited to a few low molecular weight compounds because of the extremely low permeability of human skin. Low-frequency ultrasound was shown to increase the permeability of human skin to many drugs, including high molecular weight proteins, by several orders of magnitude, thus making transdermal administration of these molecules potentially feasible. It was possible to deliver and control therapeutic doses of proteins such as insulin, interferon γ, and erythropoeitin across human skin. Low-frequency ultrasound is thus a potential noninvasive substitute for traditional methods of drug delivery, such as injections.

Transdermal drug delivery (TDD) offers several advantages over traditional delivery methods such as injections and oral administration. Compared to oral delivery, TDD avoids gastrointestinal drug metabolism, reduces elimination by liver, and provides sustained release of drugs for up to 7 days (*1*). Compared ro injections, TDD eliminates the associated pain and the possibility of infection. Theoretically, rhe transdermal route of drug administration could be advantageous in the delivery of many therapeutic proteins because (i) proteins are susceptible to gastrointestinal degradation and exhibit poor gastrointestinal uprake, (ii) proteins such as interferons are cleared rapidly from the blood (*2*) and need to be delivered at a sustained rate in order to be maintained at a high blood concentration, and (iii) transdermal devices are easier to use than injections (*1*).

Despite these advantages, few drugs and no proteins or peptides are currently adminisrered rransdermally for clinical applications, because of the low skin permeability to drugs. This low permeability is attributed to the stratum corneum (SC), the outermost skin layer that consists of flat, dead cells filled with keratin fibers (keratinocytes) surrounded by lipid bilayers. The highly ordered structure of the lipid bilayers confers an impermeable character to the SC (*3*). Several methods, which include chemical enhancers (*4*) and electricity (*5*, *6*), have been proposed to enhance transdermal drug transport, but their efficacy has been limited by the large protein size and relatively low electric charge on the proteins.

Application of ultrasound has been attempted to enhance transdermal transport of a few low molecular weight (<500) drugs across human skin (*7–10*) as well as proteins such as insulin across animal skin (*11*), a phenomenon referred to as sonophoresis. Although numerous srudies of sonophoresis have been performed (*7–11*) measurable enhancement has been reported in only a few cases (*8*, *11*). We have shown (*12*) that application of ultrasound at therapeutic frequencies (1 MHz) induces growth and oscillations of air pockets present in the keratinocytes of the SC (a phenomenon known as cavitation). These oscillations disorganize the SC lipid bilayers, thereby enhancing transdermal transport. However, application of therapeutic ultrasound does not induce transdermal transport of high molecular weight proteins. Because cavitational effects are inversely proportional to ultrasound frequency (*13*), we hypothesized that application of ultrasound at frequencies lower than that corresponding to therapeutic ultrasound may induce sufficient bilayer disorganization, so that proteins may be able to diffuse across the skin. We now report that low-frequency ultrasound can induce significant transdermal transport of proteins, including insulin (molecular weight, ~6000), interferon γ (IFN-γ) (~17,000), and erythropoeitin (~48,000).

The passive skin permeability to high molecular weight proteins, including those mentioned above, is essentially zero (below our detection limit). To assess whether application of ultrasound enhances transdermal protein flux, we measured the skin permeability to these proteins in the presence of ultrasound in vitro across human cadaver epidermis (*14*) in a Franz diffusion cell (*15*). In separate experiments, the donor compartment of the diffusion cell was filled with a solution of insulin (100 U/ml), IFN-γ (2500 U/ml), or erythropoeitin (400 U/ml). Ultrasound (20 KHz, 100-ms pulses applied every second) was applied at intensities in the range of 12.5 to 225 mW/cm² (*16*) for 4 hours by means of an ultrasound transducer that was immersed in the donor solution. The transducer (area, ~1 cm²) was oriented perpendicular ro, and placed at a distance of 1 cm from, the skin. The concentration of

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proteins in the receiver compartment was measured every hour either by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) (17). Skin permeabilities to proteins were calculated through use of the transdermal fluxes measured during the first hour (18). Ultrasound application induced significant transdermal permeation of insulin (Fig. 1A), IFN-γ, and erythropoeitin. Human skin permeability to insulin at an ultrasound intensity of 225 mW/cm^2 was 3.3×10^{-3} (±35%) cm/hour. The permeability to IFN-γ under similar ultrasound conditions was 8×10^{-4} (±22%) cm/hour, and that to erythropoeitin was 9.8×10^{-6} (±40%) cm/hour (18). At these skin permeabilities, it may be possible to deliver these proteins transdermally at a therapeutically relevant rate. For example, one could deliver an insulin dose of about 12 U/hour [a dose given three times a day to a diabetic patient (19)] from a transdermal patch with an area of 40 cm^2 (20) containing insulin at a concentration of 100 U/ml (20). Thus, 1 hour of sonophoresis performed three times a day could deliver the required daily dose of insulin to a diabetic patient. Similarly, an IFN-γ dose of $\sim 5 \times 10^6$ U/hour [a daily dose required to enhance the immune response of patients suffering from viral infection or cancer (21)] and an erythropoeitin dose of about 140 U/hour [a dose that may be given three times a day to patients suffering from severe anemia (22)] may be delivered from a similar patch by application of ultrasound (20). The ability of sonophoresis to deliver other macromolecules may be estimated on the basis of their sonophoretic skin permeability, which needs to be measured experimentally (generally decreases with increasing molecular size), and the required therapeutic dose of these macromolecules.

We further analyzed the sonophoretic enhancement of transdermal protein transport by using insulin as a model protein in vitro as well as in vivo. This analysis addresses two issues that are important in the evaluation of ultrasound as a transdermal transport enhancer: (i) the efficacy of low-frequency ultrasound in controlling transdermal flux by varying ultrasound parameters such as the intensity; and (ii) the reversibility of the transdermal transport enhancement, that is, the recovery of the barrier properties of the skin upon turning ultrasound off. The sonophoretic permeability (18) varied nearly exponentially with ultrasound intensity (Fig. 1B), probably as a result of a highly nonlinear dependence of cavitation on ultrasound intensity (23). Accordingly, ultrasound intensity might potentially be used to control transdermal insulin delivery.

Application of ultrasound under the above conditions did not appear to cause any permanent loss of the barrier properties of the skin. The transdermal insulin flux (proportional to the slope of the curves shown in Fig. 1A) 3 hours after turning the ultrasound off was statistically insignificant. To further assess the recovery of the skin barrier properties after sonophoresis, we measured water transport through the skin

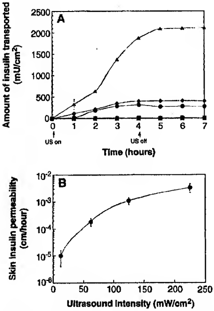

Fig. 1. (**A**) Time variation of the amount of insulin transported across human skin (in vitro) in the presence of ultrasound (20 kHz, 100-ms pulses applied every second) at 12.5 (■), 62.5 (◆), 125 (●), and 225 mW/cm^2 (▲) (n = 3 or 4; error bars, SD). (**B**) Variation of the transdermal insulin permeability (in vitro) with ultrasound intensity (20 kHz, 100-ms pulses applied every second) (n = 3 or 4; error bars, SD). The skin is impermeable to insulin at an ultrasound intensity of 0.

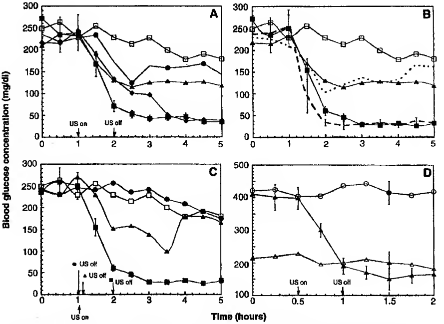

Fig. 2. (**A**) Time variation of blood glucose concentrations of 16-week-old hairless rats (IFFA, Credo, France) upon 1 hour insulin-ultrasound treatment (ultrasound was turned on at 1 hour and turned off at 2 hours) at 12.5 (●) (n = 4), 62.5 (▲) (n = 3), 125 (◆) (n = 3), and 225 mW/cm^2 (■) (n = 5) (30). Control (□), n = 5. Treatment with insulin alone or ultrasound alone did not have any effect on the blood glucose concentration. Error bars (SD) are shown for one set of data. (**B**) Comparison of blood glucose concentration of rats treated for 1 hour (from time 1 to 2 hours) with sonophoresis at 62.5 (▲) (n = 3) and 225 mW/cm^2 (n = 5) (■) and those treated with a single subcutaneous injection at time 1 hour [dashed line, 1 U (n = 3); dotted line, 100 mU (n = 3)]. A typical rat weighed about 400 g. Control (□). Error bars (SD) are shown for one set of subcutaneous and sonophoresis data. (**C**) Time variation of blood glucose concentration of hairless rats exposed to ultrasound (20 kHz, 225 mW/cm^2, 100-ms pulses applied every second) for different times. Ultrasound was turned on at 1 hour and was turned off after 1 min (●) (n = 3), 10 min (▲) (n = 3), and 1 hour (■) (n = 5) Control (□). Error bars (SD) are shown for one set of data. (**D**) Time variation of blood glucose concentration of diabetic hairless rats upon a 30-min insulin-ultrasound treatment (ultrasound was turned on at 0.5 hour and turned off at 1 hour). Diabetes was induced in the hairless rats by injection with streptozotocin (65 mg per kilogram of body weight) and was confirmed by measurements of blood glucose concentration. Sonophoresis was done as described above. Additional blood samples were taken from the tail vein, stored in heparin (~5 μg/ml), and later used to measure plasma insulin concentrations (Linco Research, St. Charles, Montana). Plasma concentration of indigenous rat insulin as well as of delivered human insulin were measured. Diabetic rats (○), normal rats (△), diabetic rats with insulin-ultrasound treatment (▲) (n = 4 per experiment; error bars, SD).

during and after ultrasound exposure (24). During sonophoresis, water permeability increased 100-fold, of which about 94 (±3%) was recovered within 2 hours after turning the ultrasound off and 98 (±1%) was recovered within 15 hours. These results suggest that application of ultrasound does not induce any long-lasting loss of the skin barrier properties.

To assess the efficacy of ultrasound in enhancing transdermal flux in an in vivo model, we performed insulin sonophoresis experiments on hairless rats (25). An intensity-dependent decrease in the blood glucose concentration was observed upon ultrasound application (Fig. 2A), indicating that low-frequency sonophoresis can effectively deliver intensity-dependent insulin doses across hairless rat skin.

We then estimated the amount of insulin penetrating the hairless rat skin during sonophoresis at various intensities (Fig. 2A). We injected known amounts of insulin in the range of 0 to 1 U subcutaneously (the most common method of insulin administration) into normal rats. The blood glucose concentrations of these rats were then compared with those of the normal rats undergoing sonophoresis. Subcutaneous injection of 100 mU and 1 U of insulin induced a decrease in the blood glucose concentration similar to that induced by sonophoresis at intensities of 62.5 and 225 mW/cm^2, respectively (Fig. 2B). These results suggest that sonophoresis delivers intensity-dependent insulin doses across the skin in the range of approximately 0 to 1 U (through an area of about 3 cm^2).

To estimate the dependence of the amount of insulin delivered on ultrasound exposure time (in vivo), we performed insulin-sonophoresis experiments on normal hairless rats exposed to ultrasound for various times in the range of 1 min to 1 hour. A 1-hour exposure resulted in a decrease of the blood glucose concentration from ~250 to ~30 mg/dl, whereas a 10-min exposure to ultrasound led to a reduction of the blood glucose concentration from about 250 to about 150 mg/dl. This result, compared with the data shown in Fig. 2B, suggests that a 1-hour ultrasound exposure delivers about 1 U of insulin, whereas a 10-min ultrasound application (225 mW/cm^2) delivers about 100 mU through an area of 3 cm^2.

Additional experiments were performed to assess whether application of ultrasound can induce sufficient insulin transport across the skin of a diabetic hairless rat so that its blood glucose concentration becomes comparable to that of normal hairless rats. Insulin-ultrasound treatment reduced the blood glucose concentration of diabetic hairless rats from ~400 to 200 mg/dl (the blood glucose concentration of normal rats) in 30 min (Fig. 2D). A corresponding change in the plasma insulin concentration was observed during sonophoresis. In normal hairless rats, the plasma insulin concentration was 101 ± 31 pM, whereas in diabetic hairless rats it was below the detection limit (34 pM). During sonophoresis performed on diabetic rats, the concentration of transdermally delivered human insulin in rat plasma was 77 (±28) pM after 30 min and 178 (±84) pM after 1 hour. No significant change in the plasma concentration of indigenous rat insulin was observed during sonophoresis.

Initial histological studies were performed to make a preliminary assessment of the safety of low-frequency sonophoresis as a penetration enhancer. These studies (26) indicated no physical damage in the skin or in the underlying muscle tissues exposed to ultrasound at all the intensities used in the experiments described above. The regions of hairless rat epidermis exposed to ultrasound were intact. Low-frequency ultrasound has been used safely in clinical applications by dentists for tooth cleaning (27). The ultrasound conditions discussed in this report (20 kHz) are similar to those used by dentists (25 to 40 kHz); however, the application time is typically longer in the case of sonophoresis.

Although our preliminary studies indicate no adverse effects of low-frequency ultrasound, further investigation into the safety of low-frequency sonophoresis is required before proceeding with clinical application. Attention should also be focused on the physiological and immunological effects of ultrasound exposure—specifically, whether a change in the method of protein administration from injection to transdermal administration will affect the body's immune response to these proteins. Furthermore, an optimal selection of ultrasound parameters, such as frequency, pulse length, and intensity, and of nonultrasonic parameters, such as ultrasound coupling medium, should be conducted to ensure a safe and efficacious application. With further research, one might envision small, pocket-size sonicators (28) carried by the patient to "inject" drugs whenever required. In addition, these devices could potentially be combined with sensors (29) that can monitor drug concentrations in the blood to formulate a self-controlled drug (insulin, for example) delivery method that might eliminate the attention required by the patient.

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16. The ultrasound intensity, I (spatial average temporal peak), was calculated from the values of the acoustic pressure amplitude, P, measured with a hydrophone (Bruel and Kjaer), by the equation $I = P^2/2\rho c$, where ρ is the water density (1 g/ml) and c is the velocity of ultrasound in water (1500 m/s).
17. The insulin concentration in the receiver compartment was measured every hour by RIA (performed at Linco Research, St. Charles, Montana). We measured the IFN-γ concentration by ELISA (Endogen), and the erythropoeitin concentration was measured by ELISA at Associated and Regional University Pathologists (Salt Lake City, UT).
18. The transdermal flux can be calculated by use of the equation $J = DM/\Delta t$, where ΔM is the amount of protein transported per unit skin area during time Δt. The skin permeability, P, can be calculated from the transdermal flux, J, during the first hour of ultrasound applicetion with the equation $P = J/\Delta C$, where ΔC is the concentration difference across the skin.
19. A typicel diabetic patient (70 kg weight) takes ~12 U of insulin three times a day (total dose ~36 U/day) [in *World Book of Diabetes in Practice*, L. P. Krall, Ed. (Elsvier, New York, 1988), pp. 160–163] If each insulin dose was to be delivered by sonophoresis in 1 hour, the required transdermal flux would be 12 U/hour. One unit of insulin corresponds to ~40 μg.
20. The transdermal patch area used in these calculations was 40 cm^2 [the area of a transdermal Fentanyl patch (Alza)]. The donor concentrations used in these calculations were as follows: Insulin, 100 U/ml (Humulin Regular, Eli Lilly); IFN-γ, 3×10^7 U/ml (typical concentration of IFN solution recommended by Genzyme); and erythropoeitin (Amgen), 3×10^5 U/ml [J. Davis, T. Arakawa, T. Strickland, D. Yphantis, *Biochemistry* **26**, 2633 (1987)].
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31. We thank M. Prausnitz for helpful suggestions, S. Lieuw and A. Patel for technical assistance, and J. Kost and M. Johnson for helpful discussions. We also thank L. Brown for assistance with diabetic hairless rats, B. Schloo for performing histological studies, and R. Gingerich for assistence with insulin measurements. Supported by NIH grant GM44884 and a gift from tha Zachery Miller Fund to R.L., and e fellowship from Schering-Plough Foundation. D.B. acknowledges the support of e NSF Presidential Young Investigator Award.

18 January 1995; accepted 5 June 1995

Parietal Contributions to Visual Feature Binding: Evidence from a Patient with Bilateral Lesions

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Neurophysiologists have documented the existence of multiple cortical areas responsive to different visual features. This modular organization has sparked theoretical interest in how the "binding problem" is solved. Recent data from a neurological patient (R.M.) with bilateral parietal-occipital lesions demonstrates that the binding problem is not just a hypothetical construct; it can be a practical problem, as rare as the selective inability to perceive motion or color. R.M. miscombines colors and shapes even under free viewing conditions and is unable to judge either relative or absolute visual locations. The evidence suggests that a single explanation—an inadequate spatial representation—can account for R.M.'s spatial judgment and feature-binding deficits.

A perplexing question in vision research is how the brain solves the "binding problem." Primate brains contain more than 20 visual areas, many of which are highly specialized for processing specific visual features (*1*). Data from human and nonhuman subjects demonstrate that object features such as color or shape are represented in hierarchical interconnected areas in a ventral visual pathway that extends from the occipital to the temporal cortex, whereas spatial features are represented in a dorsal pathway from the occipital to the parietal cortex (*2*). The wide cortical distribution of visual features, the large receptive fields of inferotemporal neurons, and the separation of spatial and object pathways lead to the question of how unified perception (or "binding") of visual objects results (*3*). Several neurophysiological studies have proposed temporally correlated neuronal activity as a mechanism for intra- and interareal coordination (*4*). Although research in cats and monkeys has been directed at exploring the neural substrate of binding, there are no documented cases of animals with binding deficits.

Treisman and Gelade (*5*) proposed that attention to spatial locations in normal human brains was necessary to properly bind the features of objects. Feature binding should therefore be disrupted by attentional overload or inaccurate spatial information. The effects of divided or reduced attention have been tested in neurologically normal people and in patient populations (*6*, *7*). When presented with brief displays of colored letters and asked to report the identity of a simultaneously presented digit, subjects experienced illusory conjunctions (ICs), as predicted. For example, if presented with a red X and a blue O, subjects sometimes confidently reported seeing a red O or a blue X. Patients with unilateral neural damage have exhibited an attentional bias away from objects in the contralesional field and have been shown to make more ICs in the contralesional than in the ipsilesional field when stimuli were briefly presented (*7*).

The binding problem seldom poses a serious challenge in nonlaboratory environments. Intact primate brains are so adept at solving the binding problem that severe limitations on processing must be imposed to observe ICs: ICs are seen in normal people only when attentional demands are high and displays are brief (200 ms) or peripheral (*6*, *8*). We have been testing, with informed consent, a 58-year-old patient (R.M.) for whom the binding problem is a significant practical challenge. R.M. has nearly symmetrical bilateral parieto-occipital lesions (Fig. 1, A to C) (*9*), with no temporal or frontal lobe involvement. R.M. did not exhibit an attentional bias for the left or right visual field but did have great difficulty in reporting where objects were located even when he directed his gaze at them. We could therefore investigate the effects of degraded spatial information on feature binding. We assessed R.M.'s ability to properly conjoin features by presenting displays containing two colored letters. His task was to report the name and color of the first letter he saw (*10*). R.M. had an IC rate of 13% even when display times were as long as 10 s and even when his attention was undivided (Fig. 2). In earlier testing sessions, his error rate was 25%, but no

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SCIENTIFIC ADVANCES

Cutaneous Tape Stripping to Accelerate the Anesthetic Effects of EMLA Cream: A Randomized, Controlled Trial

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Abstract. Most medications are not absorbed topically due to the stratum corneum barrier. While effective as a topical anesthetic, EMLA cream is absorbed slowly, delaying its effects for up to one hour, thereby limiting its usefulness. **Objective:** To determine whether removal of the cornified layer of the skin by tape stripping (TS) would allow more rapid onset of anesthesia after topical application of EMLA cream prior to IV catheterization (IVC). **Methods:** This was a prospective, randomized, controlled trial comparing the levels of pain of IVC 15 minutes after topical application of EMLA cream in patients who had TS vs patients who did not. The setting was a suburban university-affiliated ED. A convenience sample of 68 alert adult patients requiring IVC were enrolled. The primary outcomes measured were pain of IVC and pain of TS using a previously validated 100-mm visual analog scale as well as the IVC success rate. **Results:** The pain of IVC was less for TS vs control patients [29.7 mm (95% CI = 20.4 to 39.0 mm) vs 15.9 mm (95% CI = 9.1 to 22.6 mm), p = 0.017]. The mean pain of TS was 4.8 ± 7.4 mm. The IVC success rate for TS vs control patients was 91% vs 74% (p = 0.056). There were no adverse events after TS. **Conclusions:** Removal of the cornified layer of the skin resulted in a more rapid anesthetic effect of EMLA cream as evidenced by lower IVC pain scores after TS. The effectiveness of TS for enhanced absorption of other medications should be investigated. **Key words:** tape stripping; permeability; absorption; drug delivery; stratum corneum; cutaneous tape stripping. ACADEMIC EMERGENCY MEDICINE 1998; 5:1051–1056

USE OF the skin for topical delivery has many potential advantages over local injection of drugs such as local anesthetics and immunizations. In addition to eliminating the pain and fear associated with local injection,[1,2] topical delivery avoids the risk of introducing infection, eliminates the occupational risk of unintentional needlesticks, bypasses the gastrointestinal tract and liver, and may serve as a drug reservoir for sustained release.

Unfortunately, topical delivery has been limited by the relative impermeability of the skin to most medications. The major barrier to topical absorption is the stratum corneum.[3] Methods to overcome this barrier, such as the use of chemical enhancers,[4,5] electricity,[6] and ultrasound,[7,8] have met with only limited success. Tape stripping (TS) is a relatively simple method that removes layers of the stratum corneum by successive application of an adhesive tape and peeling away of the skin surface. While this method has been used to study the barrier function of the stratum corneum and the absorption of topically applied medications,[9–12] we are unaware of any studies that assessed its clinical utility.

To assess the clinical utility of TS for enhancing topical absorption of medications, we chose to evaluate its use in accelerating the anesthetic effects of EMLA cream prior to IV catheterization (IVC). While a eutectic mixture of lidocaine 2.5% and prilocaine 2.5% (EMLA cream) produces reliable anesthetic effects for various painful procedures such as venipuncture, the slow rate of absorption delays onset of its effects for nearly an hour, limiting its use in the emergent situation.[2,13]

We hypothesized that TS would speed the ab-

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Received May 29, 1998; revision received July 8, 1998; accepted July 13, 1998. Presented at the SAEM annual meeting, Chicago, IL, May 1998.
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Figure 1. Method of tape stripping. The fingers of the nondominant hand splint the surrounding skin as the cellophane tape is doubled back and peeled away with the dominant hand.

sorption and onset of effect after topical application of EMLA cream and thus allow less painful IVC than without prior stripping of the skin surface. Specifically, we compared the amounts of pain of IVC 15 minutes after topical appliction of EMLA cream with and without prior TS.

METHODS

Study Design. A randomized, controlled trial design was used to compare the levels of pain of IVC 15 minutes after topical application of EMLA cream (Astra, Mississauga, Ontario, Canada) with and without prior skin surface TS. Treatment group masking was not possible due to the nature of the intervention. This project was approved by the institutional review board. Informed consent was obtained from all study participants.

Population and Setting. The trial was conducted in the ED of the State University of New York at Stony Brook, a tertiary care center with an annual census of 50,000. A convenience sample of patients was screened by one of the investigators when he or she was present in the ED. Patients were eligible for enrollment if they were older than 18 years, required an IV catheter, were able to assess their pain, and did not require immediate IV access. Patients were excluded if they were allergic to lidocaine or prilocaine, required immediate IV access, or were unable to cooperate with pain assessments (e.g., mental disability or altered mental status).

Study Protocol. After obtaining written informed consent, the next in a series of opaque, consecutively numbered envelopes was opened to reveal the patient assignment. Envelopes were prepared by hospital pharmacy personnel not connected to the ED or enrollment process, and assignments were generated by a computerized random number program. Envelopes contained even proportions of experimental and control assignments. A structured closed-question data sheet was used to record patient demographic information. After identification of a potential hairless IV site, the investigator applied 2.5 grams of EMLA cream to the proposed area in the control patients and covered it with an occlusive dressing (Tegaderm, 3M, St. Paul, MN) for 15 minutes. Since EMLA cream is effective in most patients within 60 minutes, we believed that to be of clinical benefit, onset of anesthesia would need to be shortened to 15 minutes or less.

Prior to application of EMLA cream, the experimental patients had the outer layers of their skin surface stripped away with transparent cellophane tape (Scotch tape, 3M). Tape was applied to the IV site by an investigator and rubbed firmly to the skin surface with the investigator's index finger and then peeled away with one quick draw (Fig. 1).[14] This procedure was repeated 20 times. This number of TSs was chosen because prior studies in humans have demonstrated removal of most layers of the stratum corneum barrier after 20 strippings.[12] A new piece of tape was used for each successive TS. The direction of tape application and the direction of the stripping were the same for all 20 strippings. To reduce irritation to the adjacent skin, the surrounding skin was splinted with the investigator's nondominant hand.[12] Immediately after TS, the experimental patients rated the pain associated with TS using a previously validated 100-mm visual analog scale (VAS) marked "most painful" at the high end.[15]

The EMLA cream was wiped off 15 minutes after its application in all patients and the IV site was prepped with 70% isopropyl alcohol. IVC was performed by the investigator or the patient's practitioner with an 18- or 20-gauge catheter (Angiocath B/D, Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT) based on the clinical situation, using standard technique. Practitioners were encouraged to insert all IV catheters; however, due to the 15-minute time constraint, the investigator inserted some of the catheters. Free aspiration of blood and free flow of injected normal saline verified successful placement of the catheter. Immediately after catheterization, all the patients rated the pain of IVC using a similar VAS. Pain assessments were obtained by a research assistant not associated with the study and masked

to the study intervention. The experimental patients were also asked whether they would choose to have TS and EMLA cream prior to any future IVCs. Patients were contacted by telephone within one to two weeks and the presence of any adverse events (e.g., allergic reaction, IV site infection) was ascertained.

Measurements. The primary outcome in this study was the patient's subjective assessment of the pain associated with IVC based on the previously validated VAS. Secondary outcome measures included the rate of successful IVC, the pain associated with TS, and the development of any adverse effects associated with either TS or IVC. Minimal pain was arbitrarily defined as a pain score of ≤5 mm on the VAS.

Data Analysis. Data were entered into Access 97 (Microsoft, Inc., Redmond, WA) and imported into SPSS 7.5 for Windows (SPSS, Inc., Chicago, IL) for statistical analysis. For both pretreatment characteristics and outcomes, continuous variables were compared using a two-tailed Student's t-test. The use of parametric statistics specifically for VAS analysis has been previously validated.[16] The χ^2 test was used to compare categorical variables.

Sample size calculations were based on prior estimates of the pain associated with IVC.[17] The sample size necessary to detect a 13-mm between-group difference in pain with a power of 0.9 and α of 0.05 using a two-tailed t-test was 34 in each group. A difference of 13 mm was chosen because it has been shown to be the minimal clinically significant difference in pain scores using the VAS.[18]

To assess the simultaneous effects of IV catheter gauge and TS on the pain of IVC, a two-way analysis of variance (ANOVA) was performed using catheter gauge and TS intervention as the independent factors. There were no unplanned comparisons.

RESULTS

Of 68 patients consenting to participate in the study, 34 were randomized to the TS group intervention and 34 to the control group (Fig. 2). The mean age was 42.8 ± 20.0 years; 58% were female.

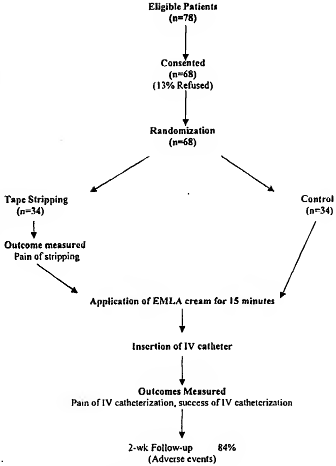

Figure 2. Study profile.

Of all the patients, 65 (96%) had IV catheters inserted in their antecubital fossae. An 18-gauge catheter was used in 46 patients (68%), while a 20-gauge catheter was used in 22 patients (32%). Table 1 lists the pretreatment demographic and clinical data for each group. At baseline there were no significant differences in age, sex, IV site, and gauge of catheter. There were significantly more nonwhite patients in the experimental group.

There was a significant between-group difference in the primary outcome measure (Table 2). Patients in the control group experienced significantly more pain than patients who had TS performed prior to IVC [29.7 mm (95% CI = 20.4 to 39.0 mm) vs 15.9 mm (95% CI = 9.1 to 22.6 mm),

TABLE 1. Pretreatment Characteristics

	All (n = 68)	Tape Stripping (n = 34)	Control (n = 34)	p-value
Age—mean (±SD)	42.8 (±20.0) yr	39.5 (±19.1) yr	47.2 (±21.6) yr	0.12
Gender—female	40 (59%)	21 (62%)	19 (56%)	0.62
Race—white	53 (78%)	22 (65%)	31 (91%)	0.03
No. with antecubital site	65 (96%)	32 (94%)	33 (97%)	0.56
No. with 18-gauge catheter	46 (68%)	24 (71%)	22 (65%)	0.57

TABLE 2. Outcome Measures*

Outcome	Tape Stripping (n = 34)	Control (n = 34)	p-value
Pain of IV catheterization—mean VAS (95% CI)	15.9 (9.1, 22.6) mm	29.7 (20.4, 39.0) mm	0.017
Pain of tape stripping—mean VAS (95% CI)	4.8 (2.2, 7.4) mm	—	—
No. with successful catheterizations	31 (91%)	25 (74%)	0.056
EMLA application time—mean (±SD)	15.1 (±1.4) min	15.1 (±0.3) min	0.91
No. with minimal pain (VAS ≤ 5 mm)	15 (44%)	6 (18%)	0.018
No. with follow-up	28 (82%)	29 (85%)	0.74

*CI indicates confidence interval; VAS, pain on visual analog scale.

p = 0.017]. The mean between-group difference in pain scores was 13.8 mm (95% CI = 2.3 to 25.3 mm). When only patients who had 18-gauge IV catheters inserted were compared, patients in the control group also experienced significantly more pain than experimental patients [32.3 mm (95% CI = 20.5 to 44.1 mm) vs 16.3 (95% CI = 7.5 to 25.0 mm), p = 0.03]. Furthermore, significantly more patients in the study group experienced minimal pain (44% vs 18%, p = 0.018). While the frequency of successful IVC was greater in patients who had TS than in controls [31 of 34 (91%) vs 25 of 34 (74%)], this trend did not quite attain statistical significance (p = 0.06). The mean pain associated with TS was 4.8 mm (95% CI = 2.2 to 7.7 mm). Of 34 experimental patients, 26 (77%) would choose TS combined with EMLA application prior to any future IVCs. While not measured for all study patients, in a small subset of patients, the time required for TS was less than 2 minutes.

From the ANOVA there was a significant effect of TS (p = 0.026), yet no effect of IV gauge (p = 0.44) or interaction effect (p = 0.75), on the pain of IVC.

Telephone follow-up was obtained for 28 of 34 (82%) experimental patients and 29 of 34 (85%) control patients. There were no adverse events associated with the study interventions in either group.

DISCUSSION

The results of this trial suggest that removal of the cornified layer of the skin (stratum corneum) by TS may be an effective method of enhancing the absorption and accelerating the onset of the anesthetic effects of EMLA cream. This is evidenced by the fact that study patients who had TS prior to application of EMLA cream and IVC experienced significantly less pain than control patients (15.9 vs 29.7 mm, p = 0.017). This difference of nearly 14 mm is both statistically and clinically significant.[18] Also, significantly more TS patients experienced minimal pain of IVC in comparison with control patients (44% vs 18%, p = 0.018). The process of TS itself caused minimal pain and discomfort and may improve the rate of successful catheterization. Furthermore, there were no adverse events associated with this procedure and it added little time to patient care.

Since topical delivery of medications offers many theoretical advantages over local injection for both patients and practitioners, researchers have evaluated multiple methods to enhance transdermal permeability. Recently Mitragotri et al. demonstrated that in comparison with standard frequencies, low-frequency ultrasound significantly increases skin permeability.[19] Using this modality they were able to normalize the glucose levels of diabetic rats by transdermal administration of insulin.[20] While these results are very encouraging, low-frequency sonophoresis has not been evaluated clinically, is expensive and cumbersome, may cause cutaneous burns,[21,22] and currently requires extended periods of application.[20] In contrast, TS is a simple and readily available method that increases skin permeability by peeling away layers of the stratum corneum. Although the effect of TS on skin permeability has been extensively investigated, we are unaware of any prior studies evaluating its clinical efficacy. While TS may be associated with temporary irritation and redness of the skin,[12] there are no known long-term risks of TS. Previous studies with TS in humans have shown that most of the permeability barrier function is restored within two to seven days.[23] While TS is followed by increased mitotic and DNA activity, this too returns to normal within six to eight days.[24] In the current study we obtained follow-up for 84% of the patients and found no evidence of any adverse events. Investigation in our laboratory found no histopathologic effects two weeks after ten to 30 TSs in young swine.[25] Thus, while TS may have transient effects on the skin, there do not appear to be any long-term sequelae, suggesting that TS is a safe procedure in humans.

While our study specifically assessed the use of TS for enhanced absorption of EMLA cream prior to IVC, we believe that its significance is far more generalizable. As noted, the current need for local injection of medications such as local anesthetics and immunizations has significant drawbacks. An alternate painless method of administration of such drugs would have obvious advantages. For ex-

ample, one of the barriers to improving the poor rate of child immunizations in many areas throughout the United States is the fear of painful injections. Thus, development of a painless alternate method to administer such medications has the potential to increase patient compliance while minimizing the associated occupational risks. Further studies will be required to refine this technique and assess whether TS will enhance the absorption of other drugs such as immunizations, analgesics, and anti-inflammatory agents.

LIMITATIONS AND FUTURE QUESTIONS

Our study has several limitations that merit discussion. First and foremost, this study was not blinded. The specific nature of the study intervention (TS) precluded masking of patients. Attempts to reduce this bias were made by encouraging practitioners blinded to the study intervention to place the IV catheters and by having trained research assistants[26] blindly administer VASs to all patients after IVC was completed. However, we cannot exclude a significant Hawthorne effect in the experimental patients that may have led to an overestimation of the benefits of TS. Future studies should attempt to fully mask the study intervention. Second, we did not attempt to measure serum levels of the local anesthetic agents to verify drug absorption. Rather, we used the patients' pain responses to IVC as a biological surrogate for topical absorption of anesthetic agents. Third, the patients evaluated adverse events over the telephone. Prior studies have demonstrated the feasibility and reliability of this method of follow-up for similar outcomes such as wound infection and cosmetic appearance.[27,28] Fourth, no attempt was made to evaluate the independent effects of TS on the primary outcome. Thus, we cannot exclude any direct effects of TS on the subsequent pain of IV catheterization. Future studies should include a TS-only control group for comparison. Fifth, follow-up was not available for all patients. Thus, it is possible that we underestimated the incidence of adverse events after TS.

Prior studies have demonstrated considerable variation in the amount of layers present in the stratum corneum between individuals and between various anatomic sites within the same individual.[29] There is also considerable variation in the number of TSs required to remove the stratum corneum based on the state of hydration of the stratum corneum and the manner of stripping.[9,12] Thus, it is possible that stripping the stratum corneum 20 times only in our study may not have been sufficient to result in substantial EMLA absorption in all of the experimental subjects. Some individuals may require significantly more strippings to have a beneficial effect on skin permeability; however, in a pilot study we found that TS became considerably more uncomfortable after 20–30 strippings (unpublished data, 1997). Thus, we chose to use 20 strippings in the present study.

Since there is a relationship between the barrier function of the skin and both its electrical resistance and its evaporative water losses, individualization of the number of TSs required in each individual patient may be possible by measuring the electrical resistance or the transepidermal water losses of the skin during the TS procedure.[9] Presumably, an increase in the electrical conductance or evaporative water losses would suggest that a significant increase in the transcutaneous permeability had been achieved. Unfortunately, this requires specialized instruments and would limit the practicality of TS in the clinical scenario. Similarly, although prior hydration of the skin for 24 hours may allow easier stripping of the stratum corneum,[12] this also would add considerable time and complexity to the procedure. Future studies should evaluate whether increasing the number of strippings and briefly hydrating the skin prior to TS would further enhance its beneficial clinical effects.

CONCLUSIONS

Our study suggests that skin surface TS is a safe and effective method for accelerating the anesthetic effect of EMLA cream prior to IVC. Further research to determine whether transcutaneous absorption of other medications also may be facilitated by TS will be key in realizing the maximum benefits of this method.

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